EXPERIENTIA

Vol. 35 - Fasc. 12 Pag. 1541-1684 15.12.1979

# GENERALIA

## The diversity of marine sterols and the role of algal bio-masses; from facts to hypothesis

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*Summary.* Modern analytical methods have revealed the great variety of marine sterols, which possess many different side chains and unsaturation patterns. Such biochemical transformations require well-defined mechanistic pathways, and there must be some 'Raison d'Etre' for a situation which has withstood evolution and adaptive changes. However, in this area, insufficient and sometimes not very substantial experimental work has made it difficult to see correlations, and hence to form a solid hypothesis. A review is now presented in which the molecules dissolved in sea water, or found in marine organisms are considered with particular reference to algal production, and to the bio-ecological significance of the main sterols.

### *1. The bio-ecological significance of sterols dissolved in sea water*

The organic matter present in the oceans, dissolved or particulate, is of interest in connection with a better understanding of the natural cycles of geochemical evolution, and also has a high bio-ecological significance for many organisms. A geographical pattern appears, concerning the distribution of organic molecules, depending on some simple factors such as the concentration in the surface microlayer, the limits of the euphotic zone, the depth, in relation to the proximity of the sea bottom, and the neighbourhood of coasts or rivers. All marine organisms are in a situation of permanent exchange with the environment, and this is indeed crucial for the smallest individuals; the oceans are like a huge culture medium for such populations. The role of organic molecules in the food chain, including the sterols (capture and metabolism), has been demonstrated[4,5] for invertrebrates such as oysters and sea anemones.

The sterol composition of sea water has been subjected to some investigations during the last decade[6-10] and has already led to surprising results. Water samples from different locations or depths have been filtered (0.45 μm) and extracted with a solvent such as chloroform (method of the French workers[8-10]), the lipids saponified, and the $\Delta_5$-sterols isolated by $SiO_2$-TLC and further analysed by GC and MS.

The method used must be kept in mind for several reasons: for instance because the choice of 0.45 μm for the millipore filters corresponds to an artifical definition of 'dissolved' molecules, or because the TLC excludes non $\Delta_5$-monohydroxylated sterols, which are thought to be minor components. This last observation is prejudicial when one considers coprostanol[11], which may be a good indicator of pollution. Furthermore, the figures obtained, which are always minimal, are given with no exact idea of the yields or losses during the whole process.

In the Atlantic, the amount of dissolved sterol[8,9] varies from 2 to 14 μg/l, with some exceptions, and in the Pacific[10], off the Guyanas and the north-eastern Brazilian coast, from 0.16 to 1.24 μg/l according to the location. Near the French coast (La Rochelle Harbour) the concentration of sterols[12] comes up to 150 μg/l, demonstrating the importance of continental contributions.

Due to the fact that cholesterol is a key metabolite for nearly all organisms, as a membrane constituent or, in Crustaceans, as an intermediate in the biosynthesis of ecdysteroids, marine dissolved sterols represent an inexhaustible source of already-synthesized molecules. The dealkylation of $C_{29}$ sterol side chains, such as in $\beta$-sitosterol or fucosterol, has also been demonstrated[4,13,14] for some marine invertebrates, and appears as a serious adaptive advantage in comparison with the usual de novo biosynthesis. But even so, the relative amount of cholesterol is determinant when it is considered as a shunt of the dealkylating enzymatic systems. In the Atlantic, dissolved cholesterol varies from 12 to 45% (from 0.24 to 7.30 μg/l) of the total amount of sterols[4], an enormous number of tons when one considers the mass of the oceans. The average concentration

(3.8 µg/l) represents approximately $6 \times 10^{12}$ molecules/ml; not a negligible quantity, even for the nonplanktonic organisms. 24-Ethyl cholesterol varies from 32 to 55% of the total dissolved sterols[4], in connection with the preponderant role of algal biomasses. Anyhow, the general situation is related to the abundance of $C_{27}$ sterols in phytoplankton[15,16], an unexpected discovery of recent years.

## *2. The diversity of marine sterols as a consequence of adaptation, a system modulated for biological purposes, and a pool of possibilities*

Model experiments performed with the marine diatom *Chaetoceros simplex calcitrans,* in order to gain more precise information about exchanges between the alga and the culture medium[17] have led to the following results. The diatoms, which contain equal quantities of cholesterol and 24-methylen cholesterol, eliminate this last sterol so that the final amount in the medium increases by 1400 times after 8 days. The reasons for the retention of cholesterol in the cells and the elimination of 24-methylen cholesterol are unknown, but could be related to the constitution and permeability of the cell walls. The fact that this last sterol is not predominant as a dissolved sterol, in spite of a high level of production by phytoplankton, may be due to its great sensitivity to oxidative degradation. The accumulation of this sterol in filtrating invertebrates, oysters for example[18], is related to the fixation by the animal of the phytoplankton sterols.

A series of $C_{26}$ sterols has been isolated from marine animals[19-22] and is also present in some algae[19,23-26], the 24-nor 5,22-cholestadien-3$\beta$-ol being predominant in most cases. The problem of the origin of these $C_{26}$ sterols is still open, as the alga *Rhodymenia palmata* which contains[19,24-26] 0.45% of 24-norcholestadienol does not incorporate[24] labelled precursors in it. A hypothesis still to be proved concerning the origin of $C_{26}$ sterols is the oxidative degradation of $C_{28}$ sterol side chains. Then, occelasterol could be a direct precursor[27], but the organisms performing such a degradation have not yet been defined and the hypothesis of an in-toto biosynthesis cannot be excluded, under certain conditions. Serols from algal cultures have often been investigated for $C_{26}$ sterols[12,16], but without success up to the present day. Models producing the $C_{26}$ sterols are still wanted in order to demonstrate the precise conditions of biosynthesis.

In many marine invertebrates, when they are sheltering colonies of unicellular algae, some unusual methylations occur in the side chains, for example for gorgosterol, acanthesterol or dinosterol[28], and there may be a connection, as animals have not so far been found to be able to alkylate sterol side chains. This is also a hypothesis waiting for model cultures, in order that the process may be demonstrated, using, of course, the same zooxanthella as are found in the animals. In some sponges, also sheltering colonies of algae, the degradation of the side chain occurs, leading to a series[29] of unexpected pregnane and androstane derivatives, and a similar situation is encountered[30] in soft corals. Italian chemists have established in sponges[31] the cyclisation of fucosterol into calysterol, a 23,24-cyclopropane sterol, and the relationship with petrosterol[32] is obvious.

The question which comes out is: how far shall we go in the isolation of new marine sterols? Is the supply unlimited, due to the possibility of minor variations in the structures? A recent paper[33] reports the isolation of about 50 sterols from one single marine organism, and using a computer-assisted structure manipulation, Djerassi et al.[34], have predicted the existence of 1,778 3-hydroxy natural sterols. As only about 100 are known, nearly all remain to be found, if Nature agrees with the computer; all this, of course, for the great pleasure of future chemists! The natural diversity of marine sterols appears to be due both to genetic controls in animals or algae, and to interactions that are connected with the history of the individual.

## *3. A return to the lipid models of biological membranes*

The now classical 'Fluid mosaic model' of the membrane proposes a static but organised structure endowed with a certain fluidity[35], in which fatty acids and sterols have important roles. The sterol esters, which are often present as liquid crystals[36,38] within a gel, modulate the cell's plasticity and general properties. The amount of free sterol in the phospholipid combs[39,40] governs the loss of electrolytes and mobile cell constituents. The biological methylation of the sterol side chains[41] diminishes the rate of their insertion in the membrane bilayers[42] in the expected order, viz. cholesterol > campesterol > $\beta$-sitosterol; and unsaturations in position 22, as in the case of stigmasterol or ergosterol, reduce the speed of insertion still further, in connection with the increase in the rigidity of the side chains. The introduction of a 24 double bond as in desmosterol has been shown to impair[43] the balance between sodium and potassium ions. And in general terms, there is an antagonistic relationship between the total amount of cholesterol in the bilayer and the efficiency of enzymatic activities.

Thus, sterols appear, following the variations of the side chain structures, to have effects on cell plasticity, membrane permeability and the exchange of solutes. The great variety of marine sterols may be connected with the specialized nature of the environment and the necessity of permanent adaptation in the face of uncommon conditions. The hypermethylation (by algae?) of sterol side chains, encountered in invertebrates sheltering colonies of unicellular algae, is very likely due to such interactions and may appear as an expression of the algal cell-host relationship, such as the preservation of structures against the host's enzymatic systems.

In all these cases, the need for model experiments on algal cultures in interacting systems is urgent, to clarify the different situations.

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# SPECIALIA

## Spiranation of phenolic diazoketones: an intermediate towards acorone synthesis

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*Summary.* Syntheses of 4-methylspiro[4.5]deca-6,9-diene-2,8-dione (**IIb**) and 1,4-dimethylspiro[4.5]deca-6,9-diene-2,8-dione (**IIc**) by spiranation of phenolic diazoketones (**Ib**) and (**Ic**) respectively are reported. Formation of (**IIc**) illustrates the first aryl participation of phenolic diazoketone prepared from higher homologue of diazomethane.

Aryl participation of simple phenolic diazoketones toward the formation of spirodienones has been developed very recently[2]. We studied this spiroannulation reaction using phenolic diazoketones having steric interference at the site of cycloalkylation centre[3]. Boron trifluoride etherate treatment of this type of diazoketones (**Ia**), where methyl groups are present in the 2 ortho positions with respect to the cyclising moiety, yielded the spiroannulated products (**IIa**) and no rearranged products (dienone-phenol type) were isolated.

In this communication we wish to report the results of cycloalkylation, under different experimental conditions, of the diazoketones, 1-diazo-4-(4-hydroxyphenyl)-2-pentanone (**Ib**) and 2-diazo-5-(4-hydroxyphenyl)-3-hexanone (**Ic**) where methyl group/groups are linked to the cyclising moiety only. 2 spirodienones, 4-methylspiro[4.5]deca-6,9-